required for the appearance of the virus-induced increment. The properties of deoxycytidine kinase in extracts of infected and uninfected cells differed in two respects: (i) the enzyme of infected cells was markedly more heat-stable, particularly in the presence of substrate; (ii) the enzyme of uninfected cells is markedly more sensitive to inhibition by the triphosphates of deoxycytidine and deoxythymidine. Taken together, these results suggest that the increment is in fact due to the synthesis of a new enzyme not present in uninfected cells.

The pool of deoxycytidine nucleotides increases after virus infection in a manner that closely parallels the increase in deoxycytidine kinase. Recent work has shown that, under certain conditions, nucleotides from cellular DNA can be reincorporated into viral DNA (P. A. J. Perera & J. M. Morrison, unpublished work). The significance of these findings will be discussed.

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Evidence for the Role of Terminal Regions of Tropocollagen in Fibrillogenesis: Specific Enzymic Degradation Studies

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The morphology of tropocollagen, precipitated as fibrils, and as the segment-long-spacing form, after digestion with pepsin for various periods, was studied in the electron microscope.

After a short digestion (1–5 h) fibrils with symmetric band patterns of the type reported by Kuhn, Kuhn & Schuppler (1964) were observed in addition to normal polarized fibrils. At 20 h tactoidal structures with both polarized and symmetric band patterns were seen. After prolonged digestion (up to 5 days) most fibrils (95%) were tactoidal with symmetric band pattern.

The segment-long-spacing form from the 20 h digest showed large dimeric tape-like aggregates. From these it could be seen that small sections had been removed from both *N*-terminal (A) and *C*-terminal (B) ends of the molecule (notation of Hodge & Schmitt, 1960).

Since pepsin is thus shown to be involved in two reactions, namely removal of fragments from both the A and B ends of the molecule, it follows that the rates of these two reactions need not be identical. Evidence for different reaction rates can, in fact, be adduced from the change in nature of the fibrillar aggregates with time, formation of symmetric fibrils

occurring more rapidly than tactoidal forms, which only arise after prolonged digestion. Tactoidal aggregates have been reported by Bard & Chapman (1968), who produced evidence for their assembly as a process of random accretion. Conversely, there is strong presumptive evidence for normal fibrillar assembly being under rigid control (J. B. L. Bard & J. Chapman, unpublished work).

Our observations could be explained if the end fragments of the molecule served different roles in fibrillogenesis, a fragment at one end being required for molecular polarization, the other for a bonding specificity related to the formation of normal fibrils.

We therefore treated tropocollagen with carboxypeptidase and leucine aminopeptidase to remove amino acids specifically from each end of the molecule.

Digestion with carboxypeptidase gave rise to polarized tactoids, whereas digestion with leucine aminopeptidase resulted in symmetric limited-diameter fibrils, suggesting that the B end is required for structural specificity whereas the A end is involved in an orientation mechanism.

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The Development of Limit Dextrinase Activity during the Germination of Cereals

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The enzyme limit dextrinase catalyses the hydrolysis of α -(1 $\rightarrow$ 6)-D-glucosidic inter-chain linkages in α -dextrins that are produced by the α -amylolytic breakdown of amylopectin. In previous studies by other workers (e.g. Kneen & Spoerl, 1948; Back, Stark & Scalf, 1948; Greig, 1963) the enzyme has been assayed by measurement of the increase in reducing power or of fermentable sugars that result from enzyme action on α -dextrins. This method of assay is subject to some dubiety if the enzyme preparation also contains α -amylase and α -glucosidases. Manners, Marshall & Yellowlees (1970) have shown that the polysaccharide pullulan is hydrolysed by cereal limit dextrinase preparations, and that this reaction can be used as a method of enzyme assay. We now report the results of such assays of protein preparations extracted from various ungerminated and germinated cereals.

Manners & Marshall (1969) have described the

changes in β -glucanase and β -glucosidase activity of protein extracts of commercial samples of barley (var. Golden Promise) during malting. The limit dextrinase activity in these extracts was initially low, and did not change significantly during steeping, although there were three- and ten-fold increases after germination for 1.5 and 3.5 days respectively. This trend continued up to 7.5 days, giving a maximum value of about a 20-fold increase. The overall rate of increase in activity was significantly lower than that of α -amylase. This conclusion differs from that of Kneen & Spoerl (1948), who reported virtually identical rates of increase.

In laboratory-scale germination experiments at 21°C, large increases in the limit dextrinase activity of extracts of rye, wheat and maize were observed after germination periods of 2–3 days. By contrast, the activity of extracts of oats, which initially had the highest activity of any of the cereals examined, did not increase markedly during germination for 4 days. The hydrolytic activity of the above extracts towards maltotriose was also examined; this did not alter greatly during the germination periods.

Since limit dextrinase represents a component of a multi-enzyme system involved in the mobilization of starch *in vivo* for metabolic purposes and the conversion of starch into fermentable sugars *in vitro*, the factors that control its formation are now being investigated.

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The Presence of an Endogenous Inhibitor of the Cholesterol Side-Chain-Cleavage System of Human Placenta

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The presence of an active cholesterol side-chain-cleavage enzyme system in human placental mitochondria was reported by Morrison, Meigs & Ryan (1965). Solubilization of the enzyme was achieved from an acetone-dried powder preparation. These workers noted that thorough treatment of the powder with ether was necessary to obtain active preparations.

The adrenal cholesterol side-chain-cleavage enzyme

and the steroid 11 β -hydroxylase systems possess similar characteristics (see Omura, Sanders, Estabrook, Cooper & Rosenthal, 1966; Simpson & Boyd, 1967a). Sharma, Forchielli & Dorfman (1962) reported the preparation of a soluble steroid 11 β -hydroxylase from bovine adrenocortical mitochondria. They observed that ultrasonic treatment followed by high-speed centrifugation resulted in increased enzymic activity as compared with the initial ultrasonically treated preparation. This suggested the presence of an 11 β -hydroxylase inhibitor associated with the insoluble particles.

We have investigated various methods for the solubilization of the cholesterol side-chain-cleavage enzyme system present in human term-placental mitochondria. The assay was performed as described by Simpson & Boyd (1967b). Native placental mitochondria converted [4-¹⁴C]cholesterol into progesterone with a 4–8% conversion in 1 h. Ultrasonic disruption of the mitochondria produced a decrease in enzymic activity. However, after ultracentrifugation (105 000g for 1 h) of the sonicated preparation, increased enzymic activity was shown in the supernatant. Similarly, freeze-drying of the sonicated mitochondria resulted in a preparation that did not show maximal activity until ultracentrifugation at 105 000g for at least 90 min. A mitochondrial acetone-dried-powder preparation exhibited a similar phenomenon.

The results suggested the presence of an enzyme inhibitor associated with the insoluble material. It was heat-stable and firmly bound to the insoluble particles, but could be extracted with chloroform-methanol (2:1, v/v). Prolonged acetone treatment of the mitochondria also resulted in removal of the inhibitor. The main components present in the acetone wash were phospholipid (mainly phosphatidylcholine and phosphatidylethanolamine) and cholesterol. The phospholipid fraction was found to be inhibitory.

The effect of authentic phosphatidylcholine and phosphatidylethanolamine on the enzymic activity was investigated. The former was found to be the more potent inhibitor of the enzymic activity. Mitochondrial phospholipid, together with other factor(s) present in the placental mitochondria, inhibited cholesterol side-chain-cleavage activity. The mechanism may involve sequestration of the cholesterol substrate in some micellar or lipoprotein form.

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